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Glucocorticoids and parental effort in tree swallows (Tachycineta bicolor)

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Introduction

All vertebrates respond to stressful situations through the release of glucocorticoid hormones (hereafter termed CORT). These hormones alter physiological processes and behavior to improve chances of survival. CORT spikes in response to unpredictable conditions such as low food availability, harsh weather conditions, or increased threat of predation (Madliger *et al.* 2015; Crespi *et al.* 2013). These behavioral and physiological adjustments may be related to ensuring future reproductive opportunities when circumstances are less than ideal for offspring survival (Breuner *et al.* 2003, Wingfield and Sapolsky 2003, Breuner 2010). Stress hormones can thus be thought of as one way organisms respond to unpredictably deleterious aspects of their environments to increase fitness (Madliger *et al.* 2015, Wingfield *et al.* 2007).

Currently, the field of stress physiology is grappling with this relationship between CORT and reproduction. The "CORT-trade-off hypothesis" assumes that while increases in CORT due to stressors will result in negative effects on investments in offspring in the short term, it would have a positive effect on individual's survival. Thus the "CORT-trade-off hypothesis" predicts that individuals trade offspring survival for their own (Zera and Harshman 2001); i.e. investing in increased chances of successful reproductive opportunities in the future (Breuner *et al.* 2003**)**. Evidence suggests that individuals are most likely to abandon their offspring when they have maximum CORT levels, possibly due to an interaction with prolactin, another hormone more explicitly linked to parental behaviors (Groscola *et al.* 2008, Angelier and Chastel 2009).

However, it has been observed that birds who are actively maintaining nestlings show unexpectedly high levels of CORT. For instance, experimentally increased CORT was shown to increase brooding behaviors in great tits (Ouyang *et al.* 2013) and naturally higher maternal CORT has been associated with higher nestling mass (Bonier *et al.* 2009). This suggests that some amount of increase in CORT may be associated with increased exhibition of parental behaviors. One reason for this might be that increased CORT may help with meeting the metabolic demands of parenthood (Bonier *et al.* 2009). More specifically, since CORT is also a metabolic hormone, the increased activity associated with parenthood (e.g. provisioning offspring) would result in an increase of CORT in that bird's system (Zera and Harshman 2001, Bonier *et al.* 2009). So, rather than CORT being the driver of increased parental behavior it may be the result of increased parental behavior.

Clearly, there is support for both hypotheses in the literature. My research tests between these two conflicting associations through the lens of CORT dosing. The amount of circulating CORT may determine the relationship between hormone level and behavior. With respect to CORT and parental effort this could determine whether that individual becomes a better or worse parent. I hypothesize that first, within the normal range of CORT concentrations in the blood, there should be a positive relationship between parental investment and CORT ("CORT-adaptation hypothesis," Bonier et al. 2009). However, when something stressful happens (e.g. bad weather conditions) which triggers a bird's stress response their CORT level would spike into stress induced ranges, causing an abandonment of reproductive behaviors as suggested by the "CORT-trade-off" hypothesis (Zera and Harshman, 2001; Bonier et al. 2009; Patterson et al. 2014). In other words, at low doses of CORT within baseline levels there is a positive relationship to parental behaviors, but at high, stress induced doses the relationship to parental behaviors becomes negative (Figure 1).

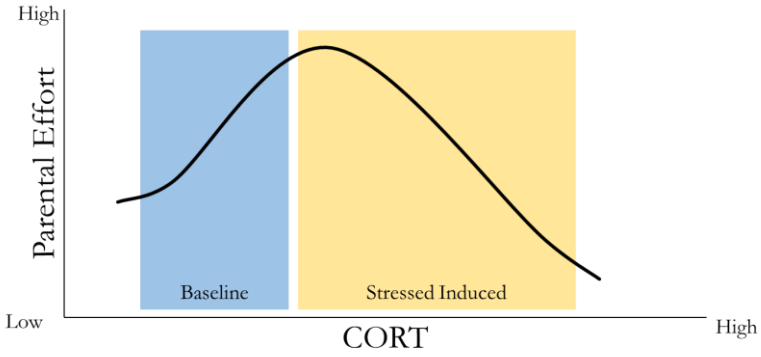


Figure 1: The hypothesized relationship between stress and parental investment if both the "CORT-adaptation" (in blue) and "CORT-fitness" (in yellow) hypotheses are supported, with a positive relationship within moderate/normal levels of CORT and a negative relationship with maximum/stress induced CORT levels.

To examine this phenomenon, I studied the parental behaviors and nestling growth rates in a free-living population of tree swallows (*Tachycineta bicolor).* Baseline and stress induced levels of CORT were measured to distinguish between moderate and extreme changes in CORT to potentially reveal the inverse relationship between the two doses and parental effort. We hypothesized that parental behaviors would be positively associated with baseline CORT and negatively associated with stress induced CORT. Our data suggest that a higher dose of stress induced CORT predicts reduced parenting. This relationship supports the predictions dose hypothesis with respect to stress induced CORT. The results are promising enough to warrant continuation of the research.

Methods

Study Population

We studied a population of tree swallows breeding in the Seeley-Swan valley in western Montana on property owned by MPG Ranch, known as MPG North. The property is about 200 acres and is near Condon, MT. With the help of Dr. Creagh Breuner, I collected data between the months of May-July 2016. There are 62 nest boxes scattered across the property; 16 boxes were occupied by tree swallows over the season. We had a final sample size of 9 due to predation, abandonment or sample loss. Tree swallows are an ideal species for addressing these questions. They readily use nest-boxes provided in appropriate habitat, and they aggressively defend their boxes, making them easy to catch repeatedly to obtain blood samples. Baseline and stress-induced levels of CORT have been previously evaluated in this species (Franceschini et al. 2008, Franceshini *et al.* 2009, Harms *et al.* 2010, Bonier *et al.* 2009), and levels are generally similar among populations

We trapped birds in their nest boxes. There was a variety of bird box types, so the method for trap doors was adapted based on the box style. Our initial strategy for capture was usually to sneak up on the nest and cover the hole with our hand, thus trapping the bird(s). If that was unsuccessful a trap door would be set up to be triggered either by a prop being knocked over as the adult entered, or by drawing a fishing line attached to the flap. Prop-style trap door was default, and in the case it was not possible due to box type we used the fishing line style. Capture was usually completed within 30 minutes if more time was needed the birds were given a break of at least a couple of hours before a second attempt.

We trapped birds twice, once during the incubation period just after egg lay and once during the nesting period on day 12-13 post-hatch. We gave the birds aluminum forest service and color bands and marked wing and tail edges with white-out at first capture (for visual identification in parental effort measures; Silvy *et al.* 2005). We also took measures of wing chord, head-bill, tarsus, mass and fat both times the birds were captured for use in calculating body condition. We took blood samples for a stress series during the nestling phase. Blood (<150µl total volume) was taken from the alar vein into a microcapillary tube using a standard stress series protocol of under 3 minutes, then at 10 minutes and 30 minutes after capture.

Behavioral Observations

We observed each nest twice for 45-minutes, once during the incubation period (usually 4 days after trapping) and once during the nestling period (day 12-13 post hatch). All birds were evaluated between 08:30-16:00, with most between 08:30-12:00. We recorded observations in an audio file which was later transcribed for data analysis. Recorded behaviors included which individuals were near the nest and which individuals entered the nest. Nest videos were also used to quantify visitation rates of individuals when nestlings were 6 days old (with two exceptions of 5 and 8 days). We recorded each nest from inside the nest box with Muvi Micro HD cameras attached inside to the roof of the nest box with Velcro. Each camera was left to record until the battery ran out (1.5-2 hours). Some recordings were unusable because of camera malfunction or disturbances (e.g. weed-whacking, rain). If the recording was unusable or due to disturbance was postponed, we recorded 1-2 days later.

Nestling Growth

We measured nestlings in 2-3 day intervals from 2 or 3 to 12 or 13 days post-hatch. We measured head bill, tarsus, wing cord, and mass. We gave each nestling a permanent marker color code for identification to keep track of individual growth rates within a nest. On day 12-13 post hatch we banded nestlings with forest service number bands.

Hormone Analysis

Concentrations of plasma corticosterone were measured using enzyme immunoassay kits from Enzo Life Sciences (Plymouth Meeting, PA, USA). All three blood samples (0, 3 and 10 minutes) from an individual were run on the same plate, all samples run over two plates. Samples were extracted twice with diethyl ether and assayed in triplicate as per Patterson *et al*. (2014). Intra-assay CofV were 3.5 and 7.7%, inter-assay CofV was 11%, and the level of detectability was 0.75 and 0.71 pg/100 ul.

Data Analysis

I explored two behavioral measures of parental effort by the females including time spent at the nest during incubation and feeding rate during nestling stage. Blood samples were only taken during the nestling period so I assumed that these measures are reflective of the incubation period as well. I calculated feed rates from the videos using the number of visits per individual over the total time observed (Bonier et al. 2011). I did not use the visual observations from the nestling phase for feeding rates because the videos were a more accurate estimate of feeding rates and individual identification. I calculated two measures of nestling growth as an indirect measure of parental effort including the peak mass reached by the nest and the peak average growth rate (K) (Remeš and Martin 2001).

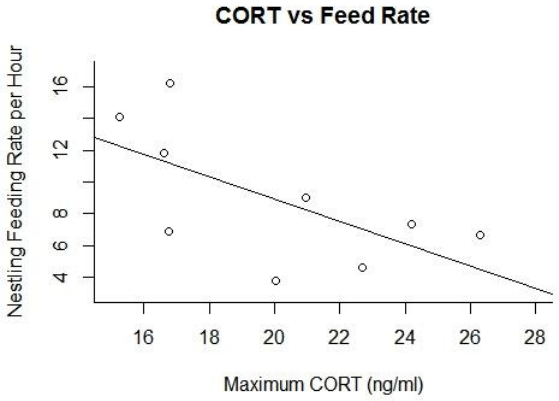
All analyses were done in the program R. I explored relationships between CORT and parental effort, examining both parental behaviors and nestling growth. Parental effort was interpreted as percent of time spent at the nest during incubation and feeding rate per hour during nestling period, both by the female only. Nesting growth was taken as peak growth rate (K) and the maximum mass of the nest as a whole. I compared each measure of parental effort with baseline CORT and stress induced CORT separately to determine if there were opposing trends based on CORT dosing (Figure 1). In case of potential confounding effects, body condition was also calculated following the methodology outlined by Peig and Greene (2009). I fit each of the relationships to a linear model chosen with AICc analysis. Because our sample size was so small (n=9), including any extra variables, such as body condition, was not helpful in clarifying existing relationships.

Results

I fit general linear regression models to each relationship between both baseline and stress induced CORT versus each measure of parental effort (percent time incubating, nestling feeding rate, peak growth rate, and maximum nest mass). Baseline CORT did not predict any behavioral measurements (Table 1, p-values > 0.3 for all). However, maximum CORT weakly predicted feeding rates (Figure 2) (β=-0.699, R^2=0.4039, p-value=0.066) and was weakly associated with growth rates (K) (Figure 3) (β=-0.039, R^2 = 0.2005, p-value = 0.194). These relationships were not statistically significant since both p-values were greater than 0.05. However, power analysis suggests that an increase of sample size of 4 for CORT vs. feeding rates and 10 for CORT vs. K could be enough for significance in both regressions. There were no relationships between maximum CORT and time incubating or maximum mass of the nest.

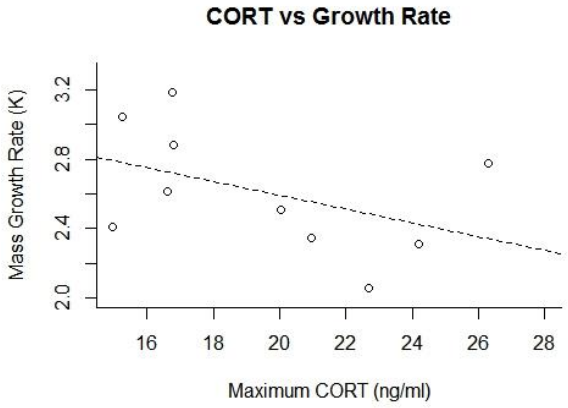
I used model selection (AICc) to determine if body condition (scaled mass body index per Peig and Green 2009) would clarify any of these relationships. However, AICc estimates only increased with this extra parameter. It's likely that our sample size was too low to include further explanatory metrics such as body condition.

Figure 2: CORT vs. Feed Rate



Maximum CORT weakly predicts lower feeding rates in females. Power analysis suggests 4 more individual needed for significance (n=9, β=-0.699, R^2 = 0.4039, p-value = 0.066)

Figure 3: CORT vs. Growth Rate



A weak trend between higher maximum CORT and the lower average peak growth rate (K) of the nest. Power analysis suggests 10 more individuals needed for significance. (n=9, β=-0.039 R^2 = 0.2005, p-value = 0.194)

Table 1

CORT	Behavior	Beta	R^2	P-value	F Statistic
Baseline	% Time Incubating	0.031	0.1278	0.310	1.173
Maximum	% Time Incubating	0.009	0.0609	0.492	0.519
Baseline	Feeding Rate	-0.061	0.0006	0.949	0.004
Maximum	**Feeding Rate**	**-0.699**	**0.4039**	**0.066**	**4.742**
Baseline	Growth Rate (K)	-0.055	0.0722	0.453	0.622
Maximum	**Growth Rate (K)**	**-0.040**	**0.2005**	**0.194**	**2.006**
Baseline	Max Mass	-0.403	0.0009	0.935	0.007
Maximum	Max Mass	0.735	0.0161	0.727	0.131

The table above shows the statistics for all the linear models each with n=9. The first two columns show the different combinations of variables and each row corresponds to that model. The two most significant relationships shown in Figures 2 and 3 are in bold.

Discussion

A distinct relationship between reproductive behavior and CORT is relatively undocumented and much disputed. The two hypotheses which were examined in this study were the CORT-Adaptation hypothesis per Bonier *et al.* (2009) and the CORT-Trade-off hypothesis (Zera and Harshman, 2001; Bonier et al. 2011; Patterson et al. 2014). In birds, there is one other hypothesis which is that stress hormones may be entirely inversely correlated with fitness which has been as summarized in the "CORT-fitness hypothesis" (Zera and Harshman 2001). The "CORT-fitness hypothesis" predicts that due to the deleterious conditions which caused the stress response, every aspect of an organism's fitness will suffer. Those conditions will negatively affect that individual's ability to allocate resources to its own survival as well as its ability to invest in offspring. It is unsurprising that the CORT response is not that simple and likely plays some positive role in coping with those conditions.

Our data suggest that there may be a negative relationship between maximum corticosterone levels and the amount of parental effort seen in tree swallows. Both negative relationships aligned with the predictions of the dosing hypothesis (Figure 1) since they were negative within stress induced ranges. However, there was no support for the baseline CORT predictions from these data. The primary reason there was little evidence of relationships was likely the small sample size (n=9) and the small amount of variance in each variable. From the 16 nests which were occupied at the start of the season, four nests were predated or abandoned and from the remaining we could collect complete data sets from only 9. Given that this was a pilot year for this study, we conclude that despite the lack of significant relationships there is enough of a trend to warrant the continuation of this study in summer 2017. The number of occupied nests was less than anticipated (hence the small sample size), however, there will be an additional field site next year likely more than doubling the sample size.

This study helps to expand understanding of the complex relationship between stress and reproductive investment. We examined the current hypothesis of how stress and reproduction interact including the "CORT-trade-off hypothesis" and the "CORT-adaptation hypothesis." It tests the relationship between them in the context of dose of CORT. Further understanding of this relationship will be important to understanding these life history trade-offs, which can have enormous influence on evolutionary trends in any population. This level of understanding of the proximal effects of potentially stressful circumstances will be increasingly important as humans influence their environments in more profound ways.

Birds are often used to understand the health of environments since they are sensitive to environmental changes. Glucocorticoids and their effects are thought to be crucial physiological mechanisms for adapting to unpredictable environmental conditions (Patterson *et al.* 2014) and that unpredictable conditions often lead to decreased reproductive success (Wingfield and Sapolsky 2003). It is important that we understand exactly how hormones cause these physiological and behavioral changes in organisms as well as what their effects may be on fitness. This study looks specifically at how stress hormones impact reproductive success, one critical component of an organism's fitness. Although there is evidence that suggests that at least some degree of elevated CORT is associated with increased parental effort, our results only support that increased CORT predicts lower parental investment.

As habitats become increasingly disturbed understanding these mechanisms may be fundamental to the preservation of biodiversity. Humans are undeniably incredible at altering our environments. Large scale impacts, such as climate change, are already causing changes in weather patterns and thus food availability. More localized impacts such as land development and noise

and waste pollution become increasingly prevalent in ecosystems as the human population continues to skyrocket. Even activities such as conservation and restoration efforts can create disturbances, despite their good intentions. Any of these processes can cause disturbances that can illicit elevated CORT levels in organisms. If humans wish to effectively reduce their impacts on their environments it is important that we understand how those impacts may affect processes like CORT and reproductive interactions.

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